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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : David F. Englert
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Title : DERIVATIVE NUCLEIC ACIDS AND USES THEREOF

Art Unit : 1634
Examiner : A. Chakrabarti

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Commissioner for Patents
Washington, D.C. 20231

RESPONSE

In response to the action mailed February 28, 2002, amend claim 1 as follows:

1. (amended) A method of analyzing a plurality of target nucleic acid sequences in a sample, the method comprising:

providing, for each target nucleic acid sequence to be analyzed, at least one probe/primer molecule which probe/primer molecule includes a region of sequence substantially complementary to a sequence in the target nucleic acid sequence and a region that is not located at either terminus of the probe/primer molecule and which includes a capture tag sequence;

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forming a reaction mixture which includes the probe/primer molecules and the target sequences under conditions such that, if a probe/primer molecule specific for a target sequence and that target sequence are both present, one or a plurality of derivative molecules having a capture tag at one or both its 3' or 5' termini, of the probe/primer molecule specific for the target sequence, is generated; and

evaluating the presence of one or more derivative molecules, each derivative molecule indicating a target nucleic acid sequence in the sample, thereby analyzing the plurality of target nucleic acid sequences in the sample.

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

July 29, 2002
Date of Deposit

Cassandra Beepot
Signature

Cassandra Beepot
Typed or Printed Name of Person Signing Certificate

Please add claims 42 and 43.

42. (new) A method of analyzing a sample of nucleic acids, the method comprising:

providing a plurality of probe molecules wherein the plurality comprises at least one probe molecule for each target nucleic acid sequence to be analyzed, the probe molecule comprising a region of sequence substantially complementary to a sequence in the target nucleic acid sequence and an internal capture tag sequence;

contacting the plurality of probe molecules to a sample of nucleic acids under conditions that allow a set of probe molecules for which a complementary sequence is present among the nucleic acids of the sample, to hybridize to the respective complementary sequence;

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cleaving the probes molecules of the set, wherein the cleavage is specific for the probe molecules that hybridize to nucleic acids of the sample and the cleavage positions the capture tag sequence of each cleaved probe molecule at a terminus of the cleaved probe molecule; and

detecting one or more of the cleaved probes, thereby analyzing the sample of nucleic acids.

43. (new) The method of claim 42 wherein each probe molecule comprises a Type IIS restriction endonuclease recognition site positioned such that cleavage of the recognition site in a double stranded DNA into which the probe molecule is incorporated generates a nucleic acid having a single-stranded overhang that includes the tag sequence. --

REMARKS

In the action mailed February 28, 2002, claims 1 to 6 and 36 to 41 have been rejected. Examiner's comments are reiterated below in bold, block-face type.

3. Claims 1-5 are rejected under 35 U.S.C. 102(e) as being anticipated by Wong (U.S. Patent 5,935,793) (August 10, 1999).

Wong teaches a method for multiplexed analysis of a plurality of target nucleic acid sequences in sample (Abstract) comprising the methods of:

providing, for each target nucleic acid sequence to be analyzed, at least one probe/primer molecule which probe/primer molecule includes a region of sequence substantially complementary

to a sequence in the target nucleic acid sequence and a region that is not located at either terminus of the probe/primer and which includes a capture tag sequence (Abstract and Figures 1A and 1B and Column 5, line 49 to column 6, line 7 and Tables 1-3);

forming a reaction mixture which includes the probe/primer molecules and the target sequences under conditions such that, if a probe/primer molecule specific for a target sequence and the target sequences are both present, one or a plurality of derivative molecules having a capture tag at one or both its 3' or 5' termini, of the probe specific for the target sequence, is generated, thereby producing a derivative nucleic acid suitable for evaluation (Figure 4 and Examples 1 and 2 and Column 12, line 6 to column 14, line 12);

evaluating the presence of one or more capture sequence tags (Figure 5 and Column 20, lines 47-60).

Applicant disagrees. Claim 1 requires forming a reaction mixture which includes the probe/primer molecules and the target sequences under conditions such that, if a probe/primer molecule specific for a target sequence and that target sequence are both present, one or a plurality of derivative molecules having a capture tag at one or both its 3' or 5' termini, of the probe specific for the target sequence, is generated. For example, prior to hybridization in the presently claimed method, the capture tag is not at a terminus of the probe/primer molecule, and after hybridization and cleavage, the tag is at a terminus. This alteration results in one or a plurality of detectable derivative molecules. Applicant respectfully submits that Wong does not disclose at least this limitation of the claim. Nothing in Wong suggests conversion of a probe/primer from an internally tagged molecule to a derivative molecule with a terminal tag in a manner dependent on the presence of the target sequence.

In general, Wong provides a method of sequencing multiple nucleic acids from a sample in parallel. For example, each of a plurality of different nucleic acids to be sequenced is provided with a unique tag at one end, the 5' end so that a reaction mixture would have, e.g,

X-TAG_A--ATTCG

X-TAG_B--AGCTA

X-TAG_C--TAGGG

Polymerase is used to amplify each of these sequences. Amplification will start at the 5' end and thus always includes the tag and will continue for various distances to create a sequence ladder. For example, with respect to the TAG_A species, one might get:

X-TAG_A--ATTC

X-TAG_A--ATT

X-TAG_A--AT

X-TAG_A--A

X can be absent or can be a primer binding site, e.g., a universal primer binding site. It is critical to note that in some embodiments of Wong, the tag is at the end (i.e., X is absent), or, in other embodiments of Wong, the tag is internal (i.e., X is present). However, in both cases, the tag is never repositioned from an internal position to a terminal position in a manner dependent on the presence of a target sequence.

After generating and separating a sequence ladder, tags are amplified from each position in the ladder and hybridized to an array. By identifying the tags at a certain position of the ladder, the nucleic acid base at that position is identified.

The Examiner alleges that Figure 4, Examples 1 and 2, and Column 12, line 6 to column 14, line 12 of Wong disclose forming a reaction mixture which includes the probe/primer molecules and the target sequences under conditions such that, if a probe/primer molecule specific for a target sequence and that target sequence are both present, one or a plurality of derivative molecules having a capture tag at one or both its 3' or 5' termini, of the probe specific for the target sequence, is generated. Applicant respectfully disagrees for at least the following reasons:

Figure 4 of Wong

Figure 4 depicts an array. According to the Brief Description of the Drawings:

> Fig. 4 shows an exemplary hybridization pattern based on the array from FIG. 3.

(6:40-41, Wong)

Figure 4 does not disclose forming a reaction mixture under conditions in which the one or a plurality of derivative molecules having a capture tag at one or both its 3' or 5' termini is generated.

Example 1 of Wong

With respect to Example 1, nucleic acids from a sample are cloned into a vector (see 20:66-21:1) Vectors are sequenced using a primer as shown in FIG. 1B. The primer includes a first universal primer binding site, a tag, and a second universal primer binding site. The sequencing process is described as follows:

> For each isolated plasmid, tag-containing sequencing fragments are generated by the Sanger sequencing method (or any functional equivalent thereof). Four separate sequencing reactions are performed in parallel for each plasmid using four different primer-tag-primers, one for each dideoxy terminator reaction (ddA, ddC, ddG, and ddT, or functional equivalents thereof). With reference to FIG. 1B, each tag-primer includes at its 3'-end, a first "universal" primer region of 20 nucleotides, for hybridizing to the plasmid DNA immediately upstream of the sample insert in the plasmid. Each tag-primer additionally includes a unique tag region of 10 nucleotides linked to the 5'-side of the first universal primer region. The tag region uniquely distinguishes each tag-primer from all others, for identifying the plasmid being sequenced and the base terminator used in the particular sequencing reaction. Finally, each tag-primer additionally includes a second "universal" primer region of 20 nucleotides linked to the 5'-side of the tag region, for later amplification of the primer-tag-primer regions. Thus, in this example, each tag-primer (also referred to as primer-tag-primer) is 50 nucleotides in length.

(21:8-27)

This sequencing process does not generate a derivative molecule having a capture tag at one or both its 3' or 5' termini. Rather Wong's tags are retained within the sequencing fragments and are flanked by a first and second universal primer region. The fragments are separated "under conditions effective to provide single-base resolution" (22:1-2). The tags are then PCR amplified using primers to the first and second universal primer binding sites from each resolved sample. These primers that bind to sites on either side of the tag (see, 22:13-23 and Fig. 1B). Indeed, if the capture tag were positioned at the terminus, the tag could not be amplified according to the described PCR process. Further, the resultant PCR products are "primer-tag-primers" (22:24) not derivative molecules having a capture tag at one or both of their termini.

PCR amplified "primer-tag-primers" are detected by hybridization to an array (22:26-51).

At no place does Example 1 suggest or disclose generating a derivative molecule from a probe/primer molecule with a capture tag sequence at one or both of its termini.

Example 2 of Wong

Example 2 uses tag-vectors which are plasmid vectors that include a tag sequence. Nucleic acids of interest are cloned near the tag sequence. The plasmid vectors are sequenced using a sequencing primer "of the form P_u -T_j –P_k" to generate sequencing fragments that include an internal tag (T_j):

> A DNA fragment mixture is cloned into a plurality of separate, different tag-vectors (V_k) of the type shown in FIG. 2A, except that universal primer region 56 is omitted, to form a plurality of vector libraries. Each vector includes a cloning site and a first vector primer sequence (P_k) which

> contains a vector-tag identifier region that is unique for each different-sequence tag-vector V_k. A clone from each library is mixed together to form a template pool in which each different vector clone is uniquely identified by the vector-identifier tag region contained in its vector primer sequence P_k '. The template pool is divided into four aliquots for performing four separate primer extension reactions, one for each terminator base-type. Each of the four aliquots is reacted with a mixture of primer-tag-primers of the form P_u -T_j -P_k to generate sequencing fragments from each different sequence clone simultaneously in the same reaction mixture, where P_u is a universal primer sequence for later PCR amplification of the primer-tag-primer region, T_j is a tag sequence for identifying the terminator base-type and sample fragment, and P_k is a vector-specific primer sequence complementary to each unique vector primer sequence P_k '. For each vector V_k, four different primer-tag-primers of the form P_u -T_j -P_k are used to generate sequencing fragments in each of four separate aliquots, such that for a given vector V_k, four different tags are used (e.g., T_1, T_2, T_3 and T_4), one for each terminator base-type, but P_u and P_k are held constant. Thus, a sequencing fragment mixture generated from first and second template pools each formed from k different vector libraries can be represented as shown in Table 2 below.

(23:10-39)

The above process does not generate a derivative molecule from a probe/primer molecule with a capture tag sequence at one or both of its termini. In fact, the tag (T_j) is internal throughout. The tag is amplified and detected as described for Example 1 of Wong.

Column 12, line 6 to column 14, line 12

Wong is directed to a method of sequencing multiple nucleic acid fragments in parallel. Different tags are attached to the respective nucleic acid fragments. Column 12 line 6 to column 14, line 12 generally teaches methods of generating sequencing fragments that include a tag.

> Conveniently, the sample or samples contain polynucleotide fragments within a selected size-range, e.g., 400-2000 nucleotides, to achieve a desired sampling frequency for effective shotgun sequencing. Fragments having selected size ranges may be prepared by standard methods, such as sonication, digestion with endonucleases and exonucleases, chemical degradation, and the like. The size range may be controlled further by subjecting the sample to agarose or polyacrylamide gel electrophoresis, size-exclusion chromatography, or other separation methods, and selecting subfractions having the desired size range.

(12:6-16)

The above passage describes generating fragments from a nucleic acid sample such as "genomic DNA, nuclear DNA, cDNA, RNA... (11:63-54)." Wong next describes cloning sample fragments to cloning vectors (see 12:13-16). There is no teaching nor suggestion to generate a derivative molecule from a probe/primer molecule.

Then Wong, describes three embodiments for attaching tags to the cloned sample fragments. In the first embodiment, cloned sample fragments are reacted with a "tag-primer."

The "tag-primer" is extended by DNA polymerase to generate a sequencing fragment that includes a tag.

> In a first embodiment for use in preparing sequencing fragments, wherein tag-primers (e.g., FIGS. 1A-1B) and a single cloning vector are employed, the sample fragments are inserted into a plurality of identical cloning vectors by standard ligation techniques, to form a mixture of sequencing vectors each containing a different sample fragment. ... Assuming that only a single label is to be used, each sequencing vector template is divided into four separate aliquots, one for each possible terminating base-type, for conducting primer extension reactions.
>
> In one embodiment, each of the four aliquots for a given vector template is reacted with a different tag-primer, and primer extension is carried out using a DNA polymerase in the presence of four deoxynucleotide triphosphates (dNTPs), with a different dideoxy terminator for each aliquot if the Sanger approach is used. Each reaction mixture produces a ladder of sequencing fragments all terminating with the same base-type, and each having the same identifier tag to indicate both the particular sample fragment and the terminator base type for the sequencing fragments produced in that reaction. Thus, for each different sequencing vector template, the product sequencing fragments contain a total of four different identifier tags for that template.

(12:39-13:10)

The above-described process of generating sequencing fragments does not include generating a derivative molecule with a capture tag at one or both of its termini from a probe/primer molecule that includes an internal capture tag.

Similarly:

> Each of the four aliquots is reacted with a plurality of tagged primers that all include (i) a first tag region that is identical among all the primers used in the aliquot, for identifying the terminating base-type of the aliquot reaction mixture, and (ii) a second, vector-tag identifier region for hybridizing to the corresponding vector-identifier tag region in each different vector clone in the aliquot to initiate primer extension. A plurality of such template pools can be prepared from the libraries and can be loaded into separate vessels (up to four vessels per template pool for the four terminator base types) for performing multiple chain extension reactions in parallel. The reaction mixtures may then be mixed together for separation on the basis of fragment length. Each sequencing fragment carries a tag sequence that identifies the source template pool, the particular vector type, and terminator base-type

(13:18-34)

The second embodiment includes inserting sample nucleic acids into vectors that include an identifier tag.

> In a second general embodiment for preparing sequencing fragments, tag-vectors are employed, such as illustrated in FIG. 2B. The sample fragments are inserted into a plurality of separate, different-sequence tag-vectors to form separate libraries of tag-vector clones. Each library contains vectors all having the same identifier tag but different sample fragment inserts. Each library is then separately plated or otherwise dispersed to produce individually isolable clones. A clone is selected from each of at least two of the plated libraries, and the selected clones are combined and are (optionally) grown together in a growth medium for a selected time, or until a

selected density has been obtained, to amplify the amount of clonal material for sequencing. The mixture of sequencing vectors is then isolated from the growth medium for use as primer extension template.

Sequencing fragments may be generated from the sequencing vector mixture using a single universal primer which is effective to initiate primer extension through the sample fragment inserts in the vectors. The primer extension reactions may be conducted together using a single aliquot of the vector mixture if four different labels attached to the 3'-terminator bases are used to distinguish the terminating base-types. Alternatively, when a four-label method is used wherein the labels are carried on the extension primer, the primer extension reactions may be separately conducted in four different aliquots, one for each base-type, which upon completion may be combined for all subsequent processing steps.

It should be noted that when tag-vectors are used in accordance with the second embodiment, primer extension beyond the identifier tag regions of the templates leads to incorporation of tag sequence complement regions near 5'-end regions of the nascent sequencing fragments. These tag sequence complements identify the sample fragments from which the sequencing fragments were derived.

(13:35-14:2, emphasis added)

The sequencing process uses a universal primer that anneals to the primer sequence **66** in FIG. 2B. During sequencing, the primer is extend through the tag sequence **62** and into the sample nucleic acid fragment inserted at the cloning site **70**. (See above, FIG. 2B, and 11:28-31). However, this process does not include generating a derivative molecule with a capture tag at one or both of its termini from a probe/primer molecule that includes an internal capture tag. Instead, the generated sequencing fragments include the tag sequence internally, 3' to the universal primer, albeit "near the 5' end."

The third embodiment is described as follows:

In a third embodiment for use in preparing sequencing fragments, tag-vectors are employed which differ from those in the second embodiment in that the primer sequence located on the 3'-side of the tag sequence is omitted. The tag-vectors in this third embodiment include (i) a cloning site, (ii) on the 3'-side of the cloning site, an identifier tag which is unique for each different-sequence tag-vector, and (iii) flanking the cloning site on one side and the identifier tag on the other side, a pair of restriction sites whose base compositions differ from that of the cloning site,

Sample fragments are inserted in the cloning sites of a plurality of separate, different-sequence tag-vectors of the type just described, to form separate libraries of tag-vector clones. As with the second embodiment, each library contains vectors all having the same identifier tag but different sample fragment inserts. Each library is then separately plated or otherwise dispersed to produce individually isolable clones. A clone is selected from each of at least two of the plated libraries, and the selected clones are combined and are (optionally) grown together in a growth medium to amplify the amount of clonal material for sequencing. The mixture of sequencing vectors is then isolated from the growth medium for forming sequencing fragments by the approach of Maxam and Gilbert.

Prior to chemical degradation, the sequencing vector mixture is digested with restriction

> endonucleases which cleave the two restriction sites flanking the tag sequence and the cloning site of the vectors, so as to excise the sample insert (with tag) from the rest of the vector. Exemplary vector constructs which may be used in this embodiment are described in Heller et al. (1991) and Church (1990; "NoC" vectors).

(14:3-34)

This embodiment also does not teach or suggest providing a probe/primer molecule which probe/primer molecule includes a region of sequence substantially complementary to a sequence in the target nucleic acid sequence and forming a reaction mixture which includes (1) the probe/primer molecules and (2) the target sequences. Rather this embodiment describes excises recombinant nucleic acids in which a tag is already fused to a sequence of interest as a single molecule. There is no species that corresponds to each of the two components that are included in the reaction mixture for the method of claim 1. In addition, there is no disclosure or suggestion that the probe/primer molecules be cleaved in a manner dependent on the presence of the target sequences.

The Examiner also asserts:

> **9. Applicant's arguments filed February 11, 2002, have been fully considered but they are not persuasive.**
>
> **Applicant argues that Wong reference does not teach the probe/primer of the claimed invention. Applicant argues that the word "modifying" or "cleaving" the tag by restriction endonuclease was not found in Wong reference and only the word "primer-tag-primer" or "tag-primer" are found. Applicant argues that because Wong has a preferred embodiment of "primer-tag-primer" or "tag-primer", Wong is limited to the preferred embodiment. This argument is not persuasive. As MPEP 2123 states "Disclosed example and preferred embodiments do not constitute a teaching away from a broader disclosure or nonpreferrred embodiments. In re Sus, 169 USPQ 423 (CCPA 1971)." MPEP 2123 also states "A reference may be relied upon for all that it would have reasonable suggest [*sic*] to one having ordinary skill the art, including nonpreferred embodiments. Merck & Co. v. Biocraft laboratories, 10 USPQ2d 1843 (Fed. Cir. 1989)" It is clear that simply because Wong has a preferred embodiment, this embodiment does not prevent the reference from suggesting broader embodiments in the disclosure and that this does not constitute a teaching away.**

Applicant disagrees with, among other things, the Examiner's characterization of Applicant's response of February 11, 2002, the Wong reference, and the law.

An anticipating reference must meet every limitation of the claim (see, e.g., MPEP 2131 and In re Schreiber, 128 F.3d 1473, 1477 (Fed. Cir. 1997)). Claim 1 is directed to a method that includes the following limitation: forming a reaction mixture which includes the probe/primer molecules and the target sequences under conditions such that, if a probe/primer molecule

specific for a target sequence and that target sequence are both present, one or a plurality of derivative molecules having a capture tag at one or both its 3' or 5' termini, of the probe specific for the target sequence, is generated.

As seen above, Applicant respectfully submits that at least this claim element is not disclosed by Wong. Moreover, neither an allegedly broader disclosure provided by Wong nor alleged non-preferred embodiments of Wong disclose or suggest the method claimed in claim 1.

The Examiner further asserts:

> **Although Wong reference uses "primer-tag-primer" or "tag-primer", the property of being modified and cleaved is inherently present in this chemically and structurally identical molecule.**

Applicant is unclear as to how the disclosure of two non-identical chemical species inherently discloses a method that requires forming a reaction mixture that generates a derivative molecule having a capture tag at a terminus from a probe/primer molecule if a probe/primer molecule specific for a target sequence and a target sequence are both present. Applicant also disagrees with the suggestion that "primer-tag-primers" are identical to the probe/primer molecules used in the method of claim 1.

The Examiner also says:

> **For example, Wong teaches that using such "primer-tag-primer" or "tag-primer" can be subject to modification and cleaving by restriction endonuclease (Column 12, line 6 to column 14, line 12).**

As seen above, Column 12, line 6 to column 14, line 12 does not teach or suggest generating a derivative molecule from a probe/primer molecule <u>if a probe/primer molecule specific for a target sequence and a target sequence are both present</u>.

Attached is a marked-up version of the changes being made by the current amendment.

Applicant asks that all claims be allowed. Enclosed is a $400 check for the Petition for Extension of Time fee. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 29/7/02

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